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Changes in the Rock-Eval signature of soil organic carbon upon extreme soil warming and chemical oxidation - A comparison

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ABSTRACT

Soil warming can increase soil organic carbon (SOC) mineralization, triggering a positive climate-carbon cycle feedback loop. Globally, many soil warming experiments have examined losses of bulk SOC, but few have assessed changes in quality. Accurate knowledge of the latter is required for an in-depth understanding and improved prediction of SOC feedback to climate change. In this study, we used Rock-Eval thermal analysis (RE6) to characterize shifts in SOC thermal stability and bulk chemistry after six years of geothermal warming by 0.6 °C, 1.8 °C, 3.9 °C, 9.9 °C, 16.3 °C, 40 °C, and 80 °C in an Icelandic grassland topsoil (0–10 cm). We also used the strong warming-induced depletion of SOC (up to 92% in the 80 °C soil) in comparisons of chemical oxidation-resistant and biogeochemically resistant SOC, which are generally assumed to be similar in nature. Sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) were used for oxidation. Warming-resistant SOC was strongly depleted in hydrocarbons and enriched in oxygen, confirming that SOC oxidation state, and thus energy content, is an important driver for biogeochemical stability. This was supported by findings that thermal stability, i.e., the amount of energy (temperature) necessary to pyrolyze or oxidize SOC, strongly increased with warming intensity. Of the 31 RE6 parameters tested, the most warming-sensitive were hydrogen index (HI, $\rho = -0.84$), oxygen index (OI_{RE6}, $\rho = 0.83$), proportion of total pyrolyzed carbon released as hydrocarbons at 200–650 °C (S2/PC, $\rho = -0.86$), and the temperature at which a certain proportion of CO_2 evolved during pyrolysis ($\rho > 0.8$). Chemical oxidation of unwarmed soil caused average relative SOC losses of 61% (NaOCl) and 91% (H_2O_2) and shifts in RE6 properties that differed strongly from warming-induced shifts at comparable SOC losses. Chemical oxidation-resistant SOC was more enriched in oxygen, but slightly enriched in hydrocarbons, and less thermostable than comparable naturally depleted SOC at the same time. A certain overlap, especially for NaOCl-treated soils, is likely, while H_2O_2-oxidized soils showed very distinct RE6 properties. We concluded that i) soil warming leads to strong shifts in SOC bulk chemistry and thermal stability and ii) H_2O_2 should be avoided in isolation of a slow SOC kinetic pool.

1. Introduction

Soil warming is observed to lead to rapid and severe losses of carbon (C) from soil organic matter (SOM) due to catalyzed microbial activity (Davidson et al., 2000; Lu et al., 2013). According to recent conservative estimates, warming-induced soil organic carbon (SOC) losses will account for 12–17% of total expected anthropogenic greenhouse gas emissions by 2050 (Crowther et al., 2016). Such losses can be expected also to lead to shifts in SOC quality, with relative enrichment of more biogeochemically resistant SOC (Knorr et al., 2005). The temperature sensitivity of older and more stabilized SOC is often reported to be higher than that of labile SOC (Lefevre et al., 2014), which is in line with kinetic theory (Davidson and Janssens, 2006). However, the large gradient in turnover times across different components of the bulk SOC pool will most likely lead to enrichment of more resistant SOC under soil warming (Conant et al., 2011). Such shifts in SOC quality have rarely been investigated, however, leading to lack of knowledge regarding the biogeochemical nature of warming-resistant SOC. This gap in research may be because i) total SOC stock changes have been the main focus of in situ warming experiments and ii) many warming

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experiments have been too short or too moderate to allow any strong shifts in SOC chemical composition and/or energetic status.

Since the SOC response to warming is one of the major uncertainties in global earth system models (Friedlingstein et al., 2006), an in-depth understanding of the mechanisms driving C losses from the soil with quantification and characterization of warming-resistant SOC are critically important. The response of different compartments or pools of SOC to warming, and inclusion of these in models, is currently being strongly debated (Conant et al., 2011), due to contradictory results in field and laboratory incubation studies and lack of mechanistic understanding.

Characterizing SOC and understanding its stabilization in the soil have always been challenging, due to the extremely diverse chemical nature of SOC (Kögel-Knabner, 2017) and to complex interactions with the mineral phase of the soil matrix (Kleber et al., 2005; Sollins et al., 1996). During the past two decades, it has been reported that selective preservation of recalcitrant organic molecules has only limited effects on the long-term persistence of organic matter in soils (Dungait et al., 2012; Rasse et al., 2006). Moreover, it is widely acknowledged, that SOM persistence is largely driven by organo-mineral interactions (Sollins et al., 1996), aggregation (Six et al., 2002), large distances between substrate and potential decomposers (Don et al., 2013), and specific environmental conditions such as energy, oxygen, and nutrient limitation (Barré et al., 2016; Fontaine et al., 2007; Schmidt et al., 2011; Wild et al., 2014).

Thermal analysis techniques such as thermogravimetry, evolved gas analysis, and differential scanning calorimetry have been applied, more or less successfully, to characterize the biogeochemical stability of SOC (Plante et al., 2009; Schiedung et al., 2017). Such studies are mainly based on the notion that the energy demand of thermal oxidation is a good proxy for the energy demand of biological oxidation (Barré et al., 2016). One emerging thermal analysis technique to characterize SOC is Rock-Eval (RE), a standard method in oil and gas exploration in sedimentary basins to assess the hydrocarbon potential of a prospect (Lafargue et al., 1998). Rock-Eval is a two-step approach, with ramped heating pyrolysis followed by ramped heating oxidation. Previous studies have demonstrated the potential of RE to characterize SOC stability and decomposition stage by relating it to respiration measurements and SOC fractions (Gregorich et al., 2015; Saenger et al., 2015), H:C ratio and O:C ratio (Espitalié et al., 1977), SOC in soil diagnostic horizons (Disnar et al., 2003) and different depth layers (Soucémarianadin et al., 2018), and long-term SOC losses in bare fallow experiments (Barré et al., 2016). Especially the latter were useful to elucidate, that RE thermal stability cannot only be linked to chemical, but also to biological stability of OM, since RE properties shifted consistently with length of bare-fallow period. Thus, OM that resisted enzymatic decay over decades was characterized by a higher RE thermal stability. Several RE indices have been developed, which can be linked to chemical SOM properties (Disnar et al., 2003) or SOC dynamics (Sebag et al., 2016). It has been concluded that the RE method is sensitive to changes associated with SOC biogeochemical processes and could have the potential for landscape-scale applications due to its time- and cost-effectiveness (Saenger et al., 2013).

Fractionation of SOM is a frequently used tool to isolate functionally distinct units of SOM, such as labile and stabile SOC pools (von von Lützow et al., 2007). The most stable pool is often isolated using strong oxidizing agents such as sodium hypochlorite (NaOCl) or hydrogen peroxide (H_2O_2) to remove the oxidizable, i.e., more labile, SOC. This is an attempt to mimic strong enzymatic decay, which is largely an oxidative process, and thus isolate a biogeochemically resistant SOC pool that is functionally linked to what is called the 'inert' or passive pool in turnover models (Zimmermann et al., 2007). For example, the remaining fraction after NaOCl oxidation is often found to be correlated with poorly crystalline minerals, which are unaffected by this form of chemical oxidation (Siregar et al., 2005) and assist in stabilizing organic matter (Mikutta et al., 2005a). In fact, NaOCl-resistant SOC has been found to be much less responsive to land-use changes (Poeplau and Don, 2013), less enriched in modern C after C3-C4 vegetation change (Poeplau and Don, 2014), and older (Helfrich et al., 2007) than bulk SOC. However, despite this general link to biogeochemical stability, it is not clear whether the approach of mimicking enzymatic decay is justified, i.e., whether chemical oxidation-resistant SOC has similar properties to long-term biogeochemically resistant SOC. This can only be directly inferred from comparing soils that are naturally extremely depleted in SOC with chemically oxidized reference soils.

In this study, we used a thermosequence of naturally warmed soil (from 0 to 80 °C), with SOC losses of up to > 80% of the initial SOC (Poeplau et al., 2017), in order to study gradual shifts in RE properties. The main objectives of the study were to 1) determine whether soil warming leads to a shift in SOC quality as revealed by RE signature, 2) identify the properties of SOC that determine its resistance to extreme soil warming, and 3) determine whether chemical oxidation with NaOCl or H_2O_2 yields SOC that is comparable to warming-resistant SOC in terms of quality.

2. Materials and methods

2.1. Study site, soil sampling and basic analysis

The study site with the natural soil warming gradients is located in southern Iceland, close to the village of Hveragerdir (64°00′01″N, 21°11′09″W, 83–168 m a.s.l.), where an earthquake on 29 May 2008 affected geothermal channels close to the surface and shifted their location, in some cases to previously unwarmed areas (O'Gorman et al., 2014). This created strong gradients in permanent soil warming within small distances (< 100 m). Since 2011, several research plots have been established on previously unwarmed soil covered with a plantation of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and unmanaged treeless grasslands dominated by common bent (*Agrostis capillaris* L.), common meadow grass (*Poa pratensis* L.), meadow horsetail (*Equisetum pratense* L.), and meadow buttercup (*Ranunculus acris* L.). In this study, only the grassland sites were investigated. Mean annual air temperature at the closest weather station (Eyrabakki, 9 km south of Hveragerdir) was 5.2 °C between 2003 and 2015 and mean annual precipitation was 1457 mm. The soil is classified as a Brown Andosol (Arnalds, 2015) and has a silty loam texture (6% clay, 53% silt, 41% sand), with pH_{H2O} 5.7, SOC content 5.4%, and C:N ratio ~10.9 in the unwarmed reference soil (0–10 cm depth) (Sigurdsson et al., 2016). Since the geothermal channels underlie and warm the bedrock, the root zone is chemically uncontaminated by geothermal waters (O'Gorman et al., 2014). On the grassland sites, five transects of permanent plots have been established along two main gradients, which represent the following average warming treatments as measured hourly from spring 2013 to spring 2015: 0 °C (unwarmed reference), 0.6 °C, 1.8 °C, 3.9 °C, 9.9 °C, and 16.3 °C. Three of these transects run downhill (unwarmed reference at the top), and two run uphill on a different slope (Sigurdsson et al., 2016). This natural randomization precludes potential effects of slope position to mask the temperature effect on SOC. For this study, we also sampled a ~40 °C warmed soil in all five transects and ~80 °C warmed soil in one transect (temperature only measured occasionally by hand). Sampling was conducted in December 2014, i.e., six and a half years after the earthquake, using a thin auger (2 cm diameter). Five auger cores (0–10 cm soil depth) were taken in direct proximity to each permanent plot and pooled to one soil sample per plot. These samples were immediately dried at 40 °C and then sieved (2 mm mesh). After ball-milling, all samples were analyzed for total C and nitrogen (N) by dry combustion in an elemental analyzer (EuroEA3024, Eurovector, Milan, Italy).

2.2. Chemical oxidation

Two commonly applied oxidizing agents were used to isolate an

oxidation-resistant SOC fraction: sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2). Both treatments were conducted using unwarmed bulk soil, in order to compare SOC that resisted chemical oxidation to SOC that resisted chronic soil warming. For NaOCl oxidation, 25 mL 6% NaOCl solution (60 g L^{-1}), adjusted to pH 8 with concentrated HCl (Zimmermann et al., 2007), were mixed with 0.5 g of bulk soil and left for 18 h at room temperature. The oxidation residue was centrifuged at 1000*g* for 15 min and washed twice with deionized water. This entire procedure was repeated twice more, to ensure complete removal of oxidizable SOC. After adding NaOCl to the soil on the second and third occasion, the centrifuge tube was vortexed to ensure complete mixture of soil and solution. After the last oxidation step, the soil was dried at 40 °C and ball-milled.

Oxidation with H_2O_2 was performed following the protocol described by Helfrich et al. (2007). To begin, 0.5 g of bulk soil was wetted with 5 mL deionized water. Then 45 mL of 10% H_2O_2 were added and the mixture was heated to 50 °C and stirred for one week. All samples were washed three times with deionized water, dried at 40 °C, and ball-milled.

2.3. Thermal analysis with Rock-Eval 6

Thermal analysis was performed with the latest version of the Rock-Eval product line (Rock-Eval 6, RE6 Turbodevice, Vinci Technologies, Nanterre, France) on a total of 46 soil samples (36 naturally warmed and unwarmed bulk soil samples and 10 chemically oxidized samples). A detailed description of the method and its latest modifications can be found in Behar et al. (2001), while the adaptations of the technique used to analyze SOM are described by Disnar et al. (2003). In brief, a sample of ~45 mg milled soil was first exposed to ramped heating pyrolysis in inert N_2 atmosphere (200–650 °C, thermal ramping rate 30° min^{-1}) and successively to thermal oxidation in a combustion oven in N_2/O_2 atmosphere (300–850 °C; thermal ramping rate 20 °C min^{-1}). Volatile and hydrocarbons (HC) were detected with flame ionization detection (FID), while evolved carbon monoxide (CO) and carbon dioxide (CO_2) were quantified with infrared (IR). During RE6 thermal analysis, five different thermograms with the following maximum temperatures for signal integration in brackets were recorded 1) volatile and pyrolyzed HC (650 °C), 2) CO evolved during pyrolysis (560 °C), 3) CO_2 evolved during pyrolysis (560 °C), 4) CO evolved during oxidation (850 °C), and 5) CO_2 evolved during oxidation (611 °C). These maximum temperatures were chosen to prevent any interference by inorganic carbon and ensure comparability with previous studies (Cécillon et al., 2018; Disnar et al., 2003). For each of the five thermograms, we derived the following five temperature parameters: T_{10}, T_{30}, T_{50}, T_{70}, and T_{90}, which corresponded to the temperature at which 10, 30, 50, 70, and 90% of the total detected gas had evolved from the sample. Signal integration of the three pyrolysis thermograms started after a 200-s isothermal pyrolysis step at 200 °C, which was performed to quantify volatile HC.

In addition to these 25 temperature parameters, we derived two standard RE indices (hydrogen index (HI) and oxygen index (OI_{RE6})) that have been shown to reflect bulk SOM chemistry (Espitalié et al., 1977; Lafargue et al., 1998). We also derived three further indices that reflect the thermostability of hydrocarbons (thermolabile hydrocarbon index (TLHC), I-Index, emphasizing the transformation of the immature organic matter fraction, and R-Index, highlighting the contribution of more refractory organic matter) (Sebag et al., 2016), where:

$$HI = \frac{HC_{200°C-650°C}}{SOC} \quad (1)$$

$$OI_{RE6} = \frac{16}{28 \times OI_{CO}} + \frac{32}{44 \times OI_{CO2}} \quad (2)$$

$$TLHC - Index = \frac{Area\ of\ HC\ pyrolysis\ thermogram\ [200^{\circ}C - 450^{\circ}C]}{Total\ area\ of\ HC\ pyrolysis\ thermogram} \quad (3)$$

$$I - Index = log\,10\left(\frac{Proportion\ of\ HC\ pyrolysis\ thermogram\ [200 - 400^{\circ}C]}{Proportion\ of\ HC\ pyrolysis\ thermogram\ [400 - 460^{\circ}C]}\right) \quad (4)$$

$$R - Index = \frac{Area\ of\ HC\ pyrolysis\ thermogramm\ [400 - 650^{\circ}C]}{Total\ area\ of\ HC\ pyrolysis\ thermogram} \quad (5)$$

where $HC_{200°C-650°C}$ is amount of hydrocarbons evolved during pyrolysis at 200–650 °C (mg HC kg^{-1}), *SOC* is the total organic carbon content in the sample (g kg^{-1}), and OI_{CO} and OI_{CO2} are the amounts of CO and CO_2 evolved during pyrolysis at 200–500 °C (CO) and 200–400 °C (CO_2) divided by the total amount of SOC in the sample. Thus OI_{RE6} is an indicator of the oxidation state of SOC and is expressed in mg O_2 g SOC^{-1}. Finally, a slight modification of HI with the focus on pyrolyzed SOC, i.e., S2/PC (mg mg^{-1}), was also derived:

$$S2/PC = \frac{HC_{200°C-650°C}}{PC} \quad (6)$$

where PC is the total C evolved during pyrolysis (mg kg^{-1}). Signal integration and calculation of the RE6 parameters was performed in R (R Development Core Team, 2010), using the packages hyperSpec (Beleites and Sergio, 2014), pracma (Borchers, 2015), and stringr (Wickham, 2015). The dataset including all 31 RE6 parameters can be downloaded from… (*add a final citation here*)….

2.4. Statistical analysis

Spearman's rank correlation coefficient (ρ) was used to evaluate the response of each of 31 different RE6 parameters to warming. This was done to identify the most warming-sensitive RE6 parameters. Furthermore, principal coordinate analysis (PCoA) or multidimensional scaling (MDS) with the centered and scaled RE6 parameters was performed to elucidate how the RE6 signature separated differently warmed and chemically treated soils. Euclidian distance was used to create the distance matrix. A priori, we reduced the dataset due to the high degree of collinearity among the 31 parameters. Parameters with a correlation coefficient of $\rho > 0.85$ were considered redundant and the one that had a less strong correlation to warming intensity was removed. Finally, a total of 11 parameters were included in the PCoA and those are marked in bold in Table 1. A non-parametric, multivariate analysis of similarity (ANOSIM) was conducted using the Euclidian distance of the 11 selected, centered and scaled indices to test for significance of differences in RE6 signatures across warming (Clarke,

Table 1
Average soil organic carbon content (SOC, g kg^{-1}), hydrogen index (HI, mg HC g C^{-1}), and oxygen index (mg O_2 g C^{-1}) with standard deviation (SD) for all soil warming intensities (°C) and oxidation agents (NaOCl = Sodium hypochlorite and H_2O_2 = hydrogen peroxide) investigated in this study. NA = not applicable.

Warming/oxidation	SOC		HI		OI_{RE6}	
Mean	Mean	SD	Mean	SD	Mean	SD
0	45	8	136	19	241	15
0.6	54.1	7	152	13	239	4
1.8	44.7	5.7	132	22	246	20
3.9	28.6	9.9	99	23	261	22
9.9	27.6	2.7	91	6	269	9
16.3	22.9	8.3	91	21	293	26
40	9	6.3	71	8	425	109
80	3.5	NA	65	NA	410	NA
NaOCl	17.7	3.2	97	6	502	40
H_2O_2	4.1	1.0	105	48	724	119

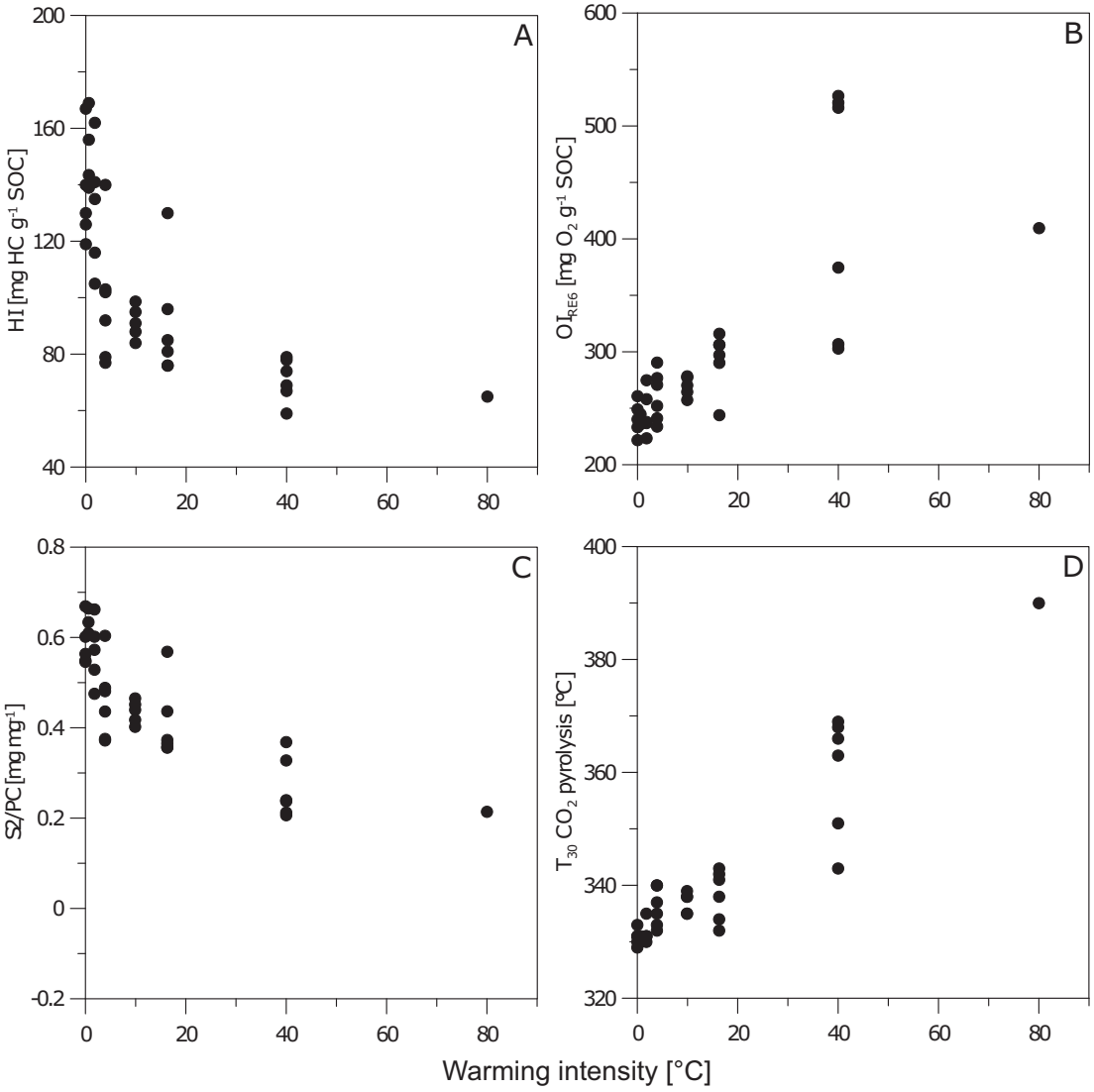

Fig. 1. A) hydrogen index (HI), B) oxygen index (OI_{RE6}), C) proportion of total pyrolyzed carbon released as hydrocarbons at 200–650 °C (S2/PC), and D) temperature at which 30% of total CO_2 obtained during pyrolysis had evolved (T_{30} CO_2 pyrolysis), all as a function of soil warming intensity.

1993). To compare naturally warmed and chemically oxidized soils, we first established correlations between total SOC content of the warmed soils and the RE6 parameters which were highly sensitive to warming, using linear and non-linear regression models. We then assessed whether the chemical oxidation-resistant SOC lay within the 95% interval of the regression model, which would indicate that biogeochemical and chemical oxidation caused similar shifts in SOC quality. For statistical analysis, we used the R package vegan (Oksanen et al., 2011).

3. Results and discussion

3.1. Effect of soil warming on thermal properties of soil organic matter

During approximately six years of geothermal warming, total SOC content in the grassland topsoil (0–10 cm) declined from 45 ± 8 g kg^{-1} (unwarmed reference soil) to 9 ± 6.3 g kg^{-1} (40 °C warmed soil). Even under sustained fresh plant-carbon input (up to 16.3 °C warmed soil), SOC was substantially depleted (Table 1) when warmed by > 0.6 °C. The increase in SOC at a low warming intensity (Table 1) indicates that also positive effects of moderate warming on SOC stocks are possible, which can be explained by stimulated net primary production due to prolonged vegetation periods and higher nitrogen mineralization rates from SOM (Rustad et al., 2001). Indeed, shoot and root biomass of the 0.6 °C warmed plots were found to be 7 and 33% higher as compared to the unwarmed grassland soil, indicating higher annual NPP (measured in July 2013, documented in the ForHot database, unpublished). The depletion in SOC was also associated with a strong shift in RE6 signatures of the remaining SOC, which reveals a link between thermal stability and biological stability in this extremely warmed soil. A previous study that investigated the biological stability of the same soils up to 16.3 °C warming using a step-wise temperature incubation showed that in situ soil warming provoked a persistent increase in metabolic quotient (microbial respiration per unit microbial biomass) of soil microbial communities, indicating increasing energy demand by soil organic matter decomposers (Marañón-Jiménez et al., 2018). Warming-persistant SOC is thus characterized by a higher biological stability than unwarmed bulk SOC. Thereby, it remains unsolved, whether transformation or selective preservation of more stable organic matter components is the prevailing cause of SOC persistence.

The classical RE6 parameters (HI and OI_{RE6}) were found to strongly decrease and increase, respectively, with increasing soil warming intensity (Fig. 1A,B). This is well in line with the dynamics of these indicators observed in long-term bare fallow treatments across Europe (Barré et al., 2016). Across different soils, Espitalié et al. (1977)

Table 2
Spearman's rank correlation coefficient (ρ) for correlations between 31 Rock-Eval thermal analysis (RE6) parameters and warming intensity. Significance: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns = non-significant. Parameters given in bold were included in the principal coordinate analysis and analysis of similarity.

RE6 parameter	ρ	*p*-Value
HI	−0.84	***
OI_{RE6}	**0.83**	***
S2/PC	**−0.86**	***
I-Index	**0.03**	**ns**
R-Index	**0.28**	**ns**
TLHC	**−0.40**	*
T_{10} HC pyrolysis	0.05	ns
T_{30} HC pyrolysis	0.29	ns
T_{50} HC pyrolysis	0.37	*
T_{70} HC pyrolysis	**0.45**	**
T_{90} HC pyrolysis	**0.64**	***
T_{10} CO pyrolysis	0.72	***
T_{30} CO pyrolysis	0.65	***
T_{50} CO pyrolysis	0.52	***
T_{70} CO pyrolysis	0.52	***
T_{90} CO pyrolysis	0.63	***
T_{10} CO_2 pyrolysis	0.81	***
T_{30} CO_2 pyrolysis	**0.88**	***
T_{50} CO_2 pyrolysis	0.86	***
T_{70} CO_2 pyrolysis	0.87	***
T_{90} CO_2 pyrolysis	0.87	***
T_{10} CO oxidation	−0.01	ns
T_{30} CO oxidation	**0.48**	**
T_{50} CO oxidation	0.73	***
T_{70} CO oxidation	0.78	***
T_{90} CO oxidation	**0.63**	***
T_{10} CO_2 oxidation	**0.03**	**ns**
T_{30} CO_2 oxidation	0.57	***
T_{50} CO_2 oxidation	0.76	***
T_{70} CO_2 oxidation	0.79	***
T_{90} CO_2 oxidation	0.80	***

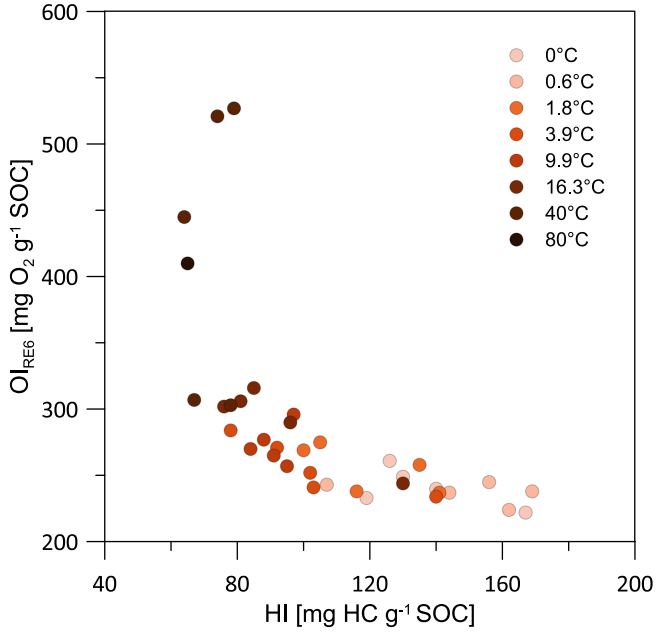

Fig. 2. Hydrogen index (HI) vs. oxygen index (OI_{RE6}) diagram.

established strong correlations of HI and H:C as well as OI and O:C ratios in SOC. The observations thus indicate that stabilized and transformed SOC is depleted in hydrogen and relatively enriched in oxygen, i.e., in a high oxidation state. This is also in line with findings that HI decreases and OI_{RE6} increases with soil depth (Sebag et al., 2016) and that there are significant positive (HI) and negative (OI_{RE6}) correlations with the proportion of particulate organic matter in forest samples (Soucémarianadin et al., 2018). Furthermore, it has been shown that the energy density of organic matter declines significantly with bare fallow duration (Barré et al., 2016), which can be explained by the high energy content of C—H bonds compared with compounds of higher nominal carbon oxidation states (LaRowe and Van Cappellen, 2011). Thus energy limitation is also likely to be an important driver for the persistence of SOC upon warming despite continued input of labile and energy-rich organic matter (up to 16.3 °C warmed soil). Following 6.5 years of geothermal warming, HI dropped from 136 ± 19 mg HC g^{-1} SOC (unwarmed reference) to 71 ± 8 mg HC g^{-1} SOC (40 °C warmed soil) (Table 1). This decline was much more pronounced, in both absolute and relative terms, than in the bare fallow treatments, in which HI dropped by < 1 mg HC g^{-1} SOC per year. This > 10-fold higher change rate is in line with observed relative enrichment of ^{13}C, another indicator of SOC transformation. Menichetti et al. (2015) detected average annual ^{13}C enrichment of 0.013‰ in bare fallow treatments, while Poeplau et al. (2017) found that bulk soil used in the present study was enriched in ^{13}C by 1‰ during six years of extreme warming (40 °C). This highlights the strong effect of warming-induced acceleration of SOC decomposition and transformation. It should however been mentioned, that this study investigated only one point in time (6.5 years after warming begun), while the bare-fallow treatments were followed over several decades with sampling every few years. Interestingly, the decline in HI seemed to level off between the 40 °C and 80 °C warming intensities, which might indicate that HI of ~70 mg HC g^{-1} SOC is an asymptotic value for this specific soil. This value is well in the range of HI values observed in a global soil sample set by (Disnar et al., 2003) for B-horizons (37–72 mg HC g^{-1} SOC), as well as the minimum value for French subsoil samples (40–80 cm, 75 mg HC g^{-1} SOC) (Soucémarianadin et al., 2018). Finally, the same value (70 mg HC g^{-1} OC) is also used as the lower threshold for assessing the petroleum potential of rocks (Sykes and Snowdon, 2002). This might indicate that a HI value ≤70 generally represents the quantity of biogeochemically persistent hydrocarbons in SOC.

For OI_{RE6}, we found a similarly strong correlation as for HI (Fig. 1B, Tables 1 and 2). Since this index describes the CO_2 and CO evolved under the inert, oxygen-free atmosphere during pyrolysis, the measured oxygen must be released from the sample and is thus an indicator of the oxidation state of SOC. Additionally, the two indices HI and OI_{RE6} and their ratio give an indication of the organic compound classes in the soil, although RE is not directly compound-specific. For example, Carrie et al. (2012) derived RE6 signatures of pure organic compounds and found an HI:OI_{RE6} ratio of 1:4 for carbohydrates and lignin and of > 4:1 for lipids, which is in accordance with the Van Krevelen diagram of atomic H:C against O:C ratio (Ohno et al., 2014). However, Carrie et al. (2012) used a slightly different RE6 protocol. In this study, we observed HI:OI_{RE6} ratios of 1:1.6 (+0.6 °C) to 1:6.3 (+80 °C) (Fig. 2). The large variation of OI_{RE6} at 40 °C was primarily related to the large variation in SOC content across replicates at this warming intensity (CV of 82%).

The S2/PC index was also among the most warming-sensitive RE6 parameters (Fig. 1C, Table 2). It describes the proportion of hydrocarbons evolved during pyrolysis at 200–650 °C of the total pyrolyzed carbon. The fact that it was slightly more strongly correlated to soil warming intensity than the similar indicator HI gives a first hint that the pyrolysis stage of RE6 might be more indicative of biological stability than the second oxidation stage. We conclude this, because the nominator of the S2/PC index is the total pyrolyzed carbon, while the nominator of HI is total SOC. The stepwise approach of RE6 including pyrolysis and oxidation might be one of the major reasons, why RE6 thermal stability indices are much better linked to biological stability than those of other methods, using solely thermal oxidation (Helfrich et al., 2010; Schiedung et al., 2017). This is underlined by the strong response of the temperature parameters of the CO_2 pyrolysis thermogram. All five parameters were strongly correlated with warming

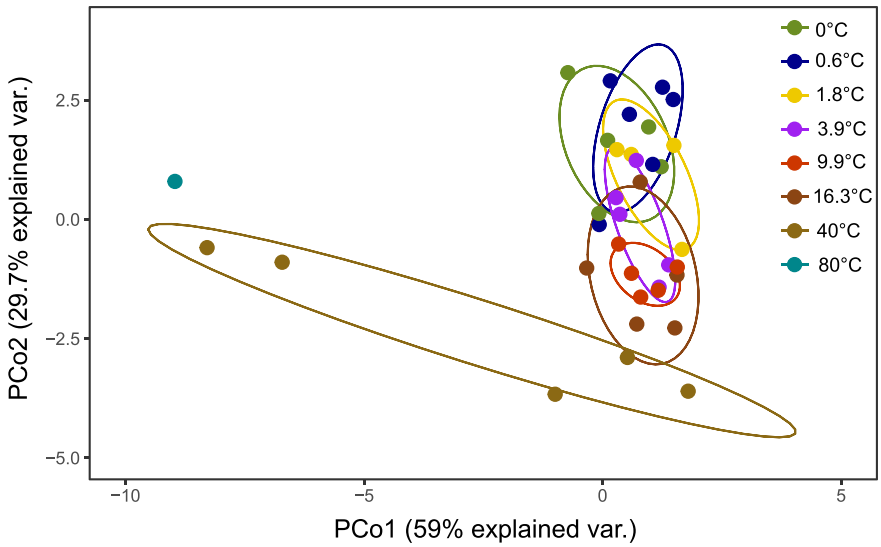

Fig. 3. Biplot of principal coordinate analysis (principal coordinate 2 (PCo2) as a function of PCo1) conducted with 11 Rock-Eval thermal analysis (RE6) parameters for soils with different warming intensity.

Table 3
Summary (R and *p* values) of analysis of similarity (ANOSIM) conducted with the reduced set of Rock-Eval thermal analysis (RE6) parameters. Lines in bold indicate the warming intensity [°C] to which a significant effect ($p < 0.05$) of warming on RE6 signatures was observed. Due to only one replicate at a warming intensity of 80 °C, no statistics could be conducted (NA = not available).

Warming	R	p
0–0.6 °C	−0.050	0.631
0–1.8 °C	−0.200	0.942
0–3.9 °C	0.016	0.370
0–9.9 °C	**0.630**	**0.009**
0–16.3 °C	**0.596**	**0.024**
0–40 °C	**0.616**	**0.008**
0–80 °C	NA	NA

intensity ($\rho > 0.8$), with the most sensitive being the temperature at which 30% of total pyrolyzed CO_2 had evolved (T_{30} CO_2 pyrolysis; Fig. 1D, Table 2). Conversely, S2-derived parameters (T_x HC pyrolysis) were weakly correlated with warming intensity. This is well in line with previous findings that several temperature parameters of the same thermogram (T_{30}, T_{50}, T_{70} CO_2 pyrolysis) are among the best predictors of proportion of centennially persistent SOC (Cécillon et al., 2018). The strong increase in these temperature parameters with increasing intensity of in situ soil warming confirms that i) thermal stability is linked to biological stability and ii) soil warming leads to a strong shift in SOC bulk chemistry and thermal stability.

The biplot of the PCoA with 11 RE6 parameters revealed that RE6 was capable of capturing warming-induced shifts in SOC quality (Fig. 3). All parameters used were either ratios or temperatures, i.e., a direct influence of SOC quantity was precluded. The on average large distance between the 40 and 80 °C warmed soils and the ≤16.3 °C warmed soils visualizes the collapse of the grassland ecosystem between 16.3 °C and 40 °C. The 40 °C warmed plots were covered by mosses only, while the 16.3 °C warmed plots were still dominated by grasses, which strongly affected organic matter input and thus also SOC quality and quantity. However, despite such strong shifts in ecosystem properties along the extreme warming gradient, as well as expected changes in mineralogy after 40° and 80 °C (Mikutta et al., 2005b), there was quite a gradual shift in RE6 signature of SOC from unwarmed to 80 °C warmed soil (Fig. 1). Despite this gradual shift, the ANOSIM revealed that significant effects of warming on RE6 signatures were detectable only after a warming intensity of 9.9 °C (Table 3).

3.2. Effect of chemical oxidation on thermal properties of soil organic matter

Chemical oxidation of the unwarmed reference soil with NaOCl and H_2O_2 depleted SOC by 61% (from 45 ± 8 to 17.7 ± 3.2 g kg^{-1}) and 91% (from 45 ± 8 to 4.1 ± 1 g kg^{-1}), respectively, which is well within the range of SOC losses observed in other studies (von von Lützow et al., 2007). In the case of NaOCl, the SOC loss was thus comparable to biological oxidation that could have occurred over six years of soil warming at between 16.3 °C and 40 °C (Table 1). The H_2O_2 treatment depleted SOC equally strong as a warming intensity of ~80 °C. The higher oxidation efficiency of H_2O_2 is in line with previous findings of residual mineral-associated SOC fractions of 5–16% for H_2O_2 and of 16–25% for NaOCl oxidation (Helfrich et al., 2007). Thus, both methods yielded quantitatively 'realistic' residual carbon fractions. Using RE6, we were able to investigate the similarity of biogeochemically resistant SOC and chemical oxidation-resistant SOC. For the most warming-sensitive RE6 parameters (Fig. 4), but also for most of the other 27 parameters (data not shown), we found that chemical oxidation-resistant SOC determined by both methods differed strongly from warming-resistant SOC. Interestingly, both HI and OI_{RE6} were higher for the chemically oxidized soils than for the biologically oxidized soils at similar SOC contents, although these indices are usually negatively correlated (Table 1). This implies that, compared with biogeochemically resistant SOC, chemical oxidation-resistant SOC is relatively enriched in hydrocarbons and oxygen at the same time. The shift in OI_{RE6} was especially pronounced in the H_2O_2 treatment, in which the average OI_{RE6} increased from 241 ± 15 to 724 ± 119 mg O_2 g SOC^{-1}, while the highest OI_{RE6} value observed for naturally warmed soils was 425 ± 109 (Table 1). The high oxidation state of SOC in H_2O_2-treated samples might be related to the fact that H_2O_2 peroxidation transforms a certain fraction of SOC into oxalate (Farmer and Mitchell, 1963). This oxalate, which is an artefact of the chemical treatment, can react with sesquioxides and lead to significant further destabilization of SOC. While we cannot entirely preclude a direct impact of oxalate on the RE6 signature, we hope that washing the sample three times with deionized water has removed it from the soil.

At the same time, high H_2O_2 resistance of low-molecular weight aliphatic hydrocarbons has been reported (Xie and Barcelona, 2003), which might explain the relatively elevated HI of the H_2O_2-treated soils

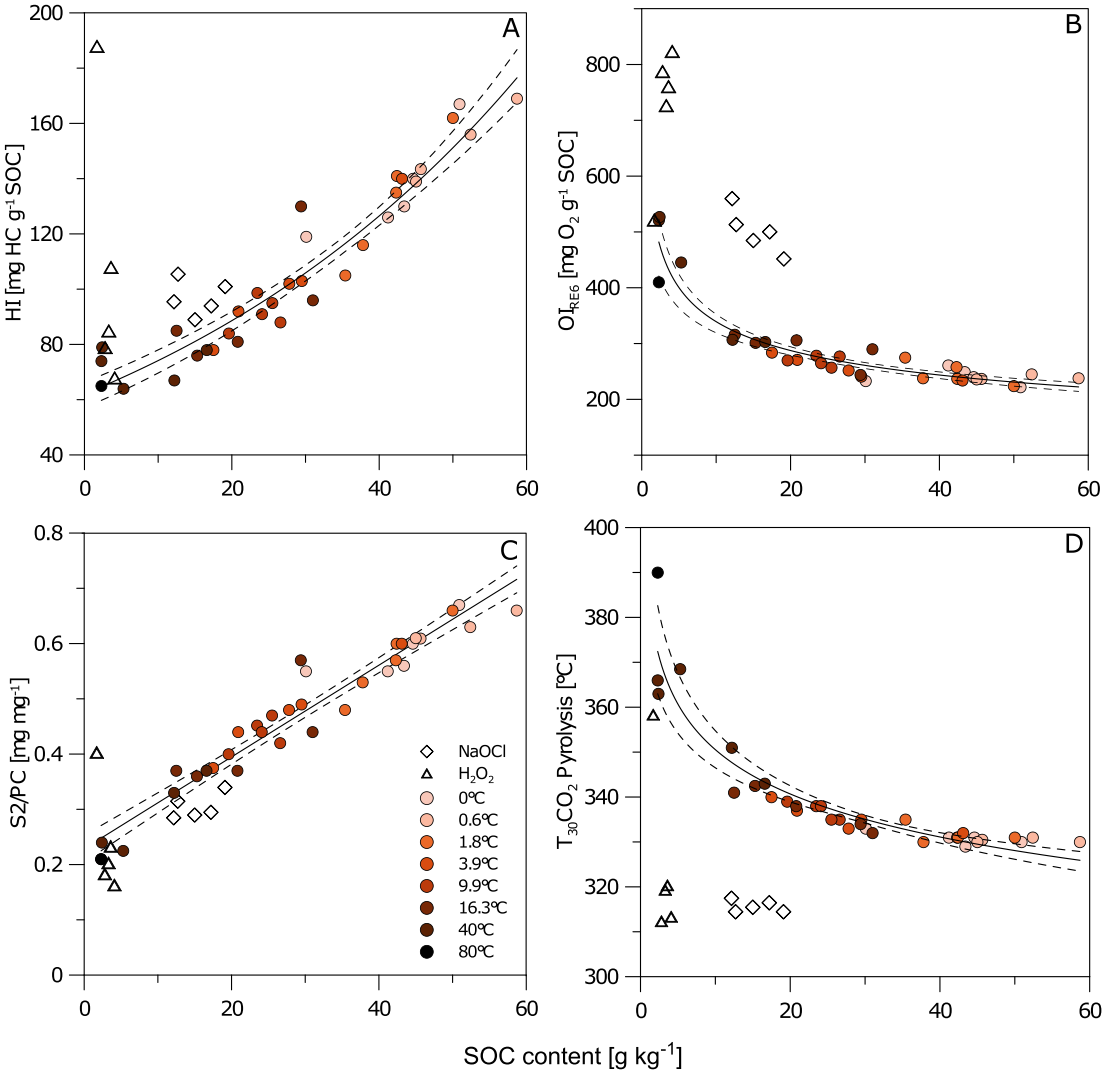

Fig. 4. A) hydrogen index (HI), B) oxygen index (OI_{RE6}), C) proportion of total pyrolyzed carbon released as hydrocarbons at 200–650 °C (S2/PC), and D) temperature at which 30% of the total CO_2 obtained during pyrolysis had evolved (T_{30} CO_2 pyrolysis), all as a function soil organic carbon (SOC) content, for soils with different warming intensity and chemically oxidized soils. Regression lines are for the chemically untreated soils (solid) and their 95% confidence intervals (dashed).

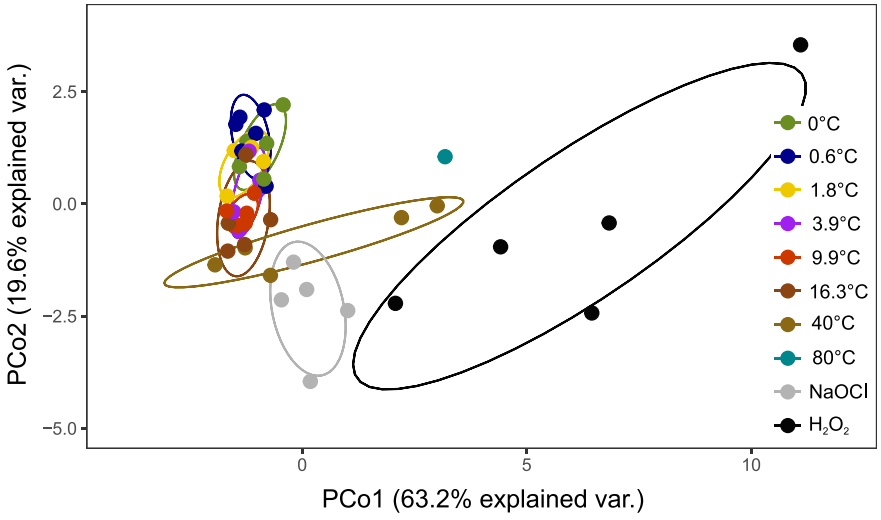

Fig. 5. Biplot of principal coordinate analysis (principal coordinate 2 (PCo2) as a function of PCo1) conducted with 11 Rock-Eval thermal analysis (RE6) parameters for soils with different warming intensity and chemically oxidized soils (H_2O_2 = hydrogen peroxide, NaOCl = sodium hypochloride).

in this study. Relative depletion of aromatic compounds and relative enrichment of aliphatic compounds upon H_2O_2 oxidation has observed in several studies (Leifeld and Kögel-Knabner, 2001; Schulten et al., 1996). It is also remarkable that, despite the higher oxygen content of the chemically oxidized samples, the thermal stability of the pyrolyzable fraction of chemically oxidized SOC was much lower (Fig. 3D; T_{30} CO_2 Pyrolysis as an example).

Microporous Al- and Fe-oxides and other poorly crystalline constituents are known to stabilize SOC against NaOCl oxidation and microbial decay (Mikutta et al., 2005a; Siregar et al., 2005). However, the strongly different RE6 signature of NaOCl-treated and naturally warmed soils in the present study indicates that there is only a limited overlap of resistant SOC, such as SOC in organo-mineral complexes. Similarly to H_2O_2 oxidation, Westerhoff et al. (2004) found that aromatic compounds of natural organic matter are more reactive with aqueous chlorine than aliphatic compounds, which might partly explain the different RE6 signature. Overall, the shift in SOC quality with NaOCl was less extreme than with H_2O_2 oxidation, as indicated by the smaller distance between the warmed soils and the oxidized samples in the PCoA biplot (Fig. 5). Thus, NaOCl oxidation seems to yield a fraction that comes closer to biogeochemically resistant SOC than H_2O_2 oxidation. However, the total SOC loss induced by NaOCl oxidation did not yield a fraction that was biogeochemically persistent as a whole, since SOC loss at a warming intensity of 40 °C was more pronounced (Table 1). Moreover, thermal stability as a proxy for biogeochemical stability was lower than for similarly SOC-depleted warmed soils (Fig. 3D). Thus, at least a certain part of the oxidation-resistant SOC seems to be more thermolabile than warming-resistant SOC. This fits previous observations, that this fraction responds in the same direction as the bulk SOC to a gradient of geothermal warming (Poeplau et al., 2017), land-use change (Dondini et al., 2009; Poeplau and Don, 2013; Poeplau and Don, 2014), and long-term bare fallow (Lutfalla et al., 2014). Spohn and Giani (2011) even found a stronger decline in NaOCl-resistant SOC than bulk SOC upon cultivation of pastures. These findings indicate that the chemically isolated SOC fraction does not resemble a homogeneous 'passive' or biogeochemically 'inert' SOC pool, as assumed in many established SOC turnover models (Coleman and Jenkinson, 1996; Taghizadeh-Toosi et al., 2014). This assumption has in fact been questioned previously (Lutfalla et al., 2014).

It could not be clarified to what extent SOC was modified by chemical oxidation, but comparing the oxidation-residues to naturally depleted SOC revealed a much stronger shift in RE6 signature when H_2O_2 was used. It is likely, that SOC compartments that resisted six years of extreme soil warming have been oxidized within several days of chemical treatment and that other compartments might have resisted chemical oxidation but were broken down by enzymes upon warming. Furthermore, the variability of several RE6 parameters in the H_2O_2-oxidized samples was extremely high as compared to NaOCl oxidized samples (Fig. 5, Table 1), indicating a limited reproducibility of the method. If isolation of an 'inert' or centennially persistent SOC pool is not the goal of a fractionation attempt, and only a 'slow-cycling' and somewhat 'older' SOC pool is to be isolated, the results of the present study indicate that use of NaOCl is preferable.

3.3. Soil organic matter fractionation or thermal analysis?

As shown in this and several other studies, Rock-Eval thermal analysis has the potential to characterize SOC quality and its response to environmental change. For example, RE6 parameters have been found to correlate with the labile particulate organic matter fraction and easily mineralizable SOC (Saenger et al., 2015; Soucémarianadin et al., 2018). Furthermore, despite a strong soil matrix effect on RE6 parameters, together they have been found to be a good predictor for the size of the centennially persistent SOC pool, even in soils outside a training sample set (Cécillon et al., 2018). Thus, two kinetically important pools, i.e., the most labile and the most stable SOC, have been shown to be predictable with RE6 under pedo-climatic conditions similar to those of a calibration sample set, which is currently restricted to the long-term bare fallow network in central Europe. Use of the method has also resulted in improved understanding of the nature of centennially persistent SOC, which has been found to be thermally stable and strongly energy-depleted (Barré et al., 2016). Finally, RE6 is more cost- and time-effective than other methods for SOC characterization, including fractionation. However, the present study provides an excellent example that SOM fractionation methods yield mechanistic insights on SOC dynamics that cannot be observed with thermal analysis methods such as RE6. Poeplau et al. (2017) isolated five different SOM fractions from all samples analyzed by RE6 in the present study, and detected loss of aggregation with increasing warming intensity, with the stable aggregate fraction > 63 µm being depleted in SOC and mass. This indicates an important mechanism triggering loss of SOC upon warming that can be further linked to changes in other biotic and abiotic drivers of aggregate formation and stabilization, such as soil moisture, fine root density, and mycorrhiza abundance. In particular, physical fractionation, i.e., by size and density, can be very helpful in understand and monitoring the transformation of plant litter into the most stable forms of SOC (Cotrufo et al., 2015). In contrast, interpretation of RE6 signatures is much less straightforward (Disnar et al., 2003; Sebag et al., 2016). Another advantage of SOM fractionation is that any kind of further analysis, including RE6, can be applied to the isolated fractions. Depending on the research question, a combination of both approaches might thus yield important further insights into SOM cycling.

4. Conclusions

This study is among the first to provide insights on how soil warming changes SOC bulk chemistry and thermal stability. We found that depletion of SOC and hydrocarbons increased with warming intensity and that resistant SOC was strongly enriched in oxygen, confirming energy limitation as a major driver for SOC persistence. Thermal stability also increased with warming intensity, indicating that thermal stability was a good proxy for biogeochemical stability. Comparison of naturally depleted and chemically oxidized SOC revealed large differences in RE6 thermal properties, which questions the notion of chemically isolating a homogeneous SOC fraction of biogeochemical relevance. Of the two different oxidation agents tested, NaOCl treatment of soils gave a RE6 signature that was much closer to naturally-depleted soils than H_2O_2 oxidization, indicating a certain overlap of biogeochemical and NaOCl-resistant SOC. Isolating a biogeochemically resistant or slow-cycling SOC pool by means of H_2O_2 oxidation should thus be avoided.

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References

Arnalds, O., 2015. The Soils of Iceland. Springer.
Barré, P., Plante, A.F., Cécillon, L., Lutfalla, S., Baudin, F., Bernard, S., Christensen, B.T., Eglin, T., Fernandez, J.M., Houot, S., 2016. The energetic and chemical signatures of persistent soil organic matter. Biogeochemistry 130 (1–2), 1–12.
Behar, F., Beaumont, V., Penteado, H.D.B., 2001. Rock-Eval 6 technology: performances and developments. Oil Gas Sci. Technol. 56 (2), 111–134.
Beleites, C., Sergio, V., 2014. hyperSpec: a Package to Handle Hyperspectral Data Sets in R. R Package Version 0.98-20140523.
Borchers, H.W., 2015. pracma: Practical Numerical Math Functions. R Package Version 1.8.8.
Carrie, J., Sanei, H., Stern, G., 2012. Standardisation of Rock–Eval pyrolysis for the analysis of recent sediments and soils. Org. Geochem. 46, 38–53.
Cécillon, L., Baudin, F., Chenu, C., Houot, S., Jolivet, R., Kätterer, T., Lutfalla, S., MacDonald, A., Van Oort, F., Plante, A.F., 2018. A model based on Rock-Eval thermal analysis to quantify the size of the centennially persistent organic carbon pool in

temperate soils. Biogeosciences 15 (9), 2835.
Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Austral Ecol. 18 (1), 117–143.
Coleman, D.C., Jenkinson, D.S., 1996. RothC-26.3 - a model for the turnover of carbon in soil. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), Evaluation of Soil Organic Matter Models Using Existing Long-Term Datasets. NATO ASI Series I. Springer, Berlin, pp. 237–246.
Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., 2011. Temperature and soil organic matter decomposition rates–synthesis of current knowledge and a way forward. Glob. Chang. Biol. 17 (11), 3392–3404.
Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8 (10), 776–779.
Crowther, T.W., Todd-Brown, K.E.O., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B., Snoek, B.L., Fang, S., Zhou, G., Allison, S.D., Blair, J.M., Bridgham, S.D., Burton, A.J., Carrillo, Y., Reich, P.B., Clark, J.S., Classen, A.T., Dijkstra, F.A., Elberling, B., Emmett, B.A., Estiarte, M., Frey, S.D., Guo, J., Harte, J., Jiang, L., Johnson, B.R., Kröel-Dulay, G., Larsen, K.S., Laudon, H., Lavallee, J.M., Luo, Y., Lupascu, M., Ma, L.N., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L.L., Schmidt, I.K., Sistla, S., Sokol, N.W., Templer, P.H., Treseder, K.K., Welker, J.M., Bradford, M.A., 2016. Quantifying global soil carbon losses in response to warming. Nature 540, 104.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440 (7081), 165–173.
Davidson, E.A., Trumbore, S.E., Amundson, R., 2000. Biogeochemistry: soil warming and organic carbon content. Nature 408 (6814), 789–790.
Disnar, J.-R., Guillet, B., Kéravis, D., Di-Giovanni, C., Sebag, D., 2003. Soil organic matter (SOM) characterization by Rock-Eval pyrolysis: scope and limitations. Org. Geochem. 34 (3), 327–343.
Don, A., Rödenbeck, C., Gleixner, G., 2013. Unexpected control of soil carbon turnover by soil carbon concentration. Environ. Chem. Lett. 1–7.
Dondini, M., Hastings, A., Saiz, G., Jones, M.B., Smith, P., 2009. The potential of Miscanthus to sequester carbon in soils: comparing field measurements in Carlow, Ireland to model predictions. Glob. Change Biol. Bioenergy. 1 (6), 413–425.
Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob. Chang. Biol. 18 (6), 1781–1796.
Espitalié, J., Laporte, J.L., Madec, M., Marquis, F., Leplat, P., Paulet, J., Boutefeu, A., 1977. Méthode rapide de caractérisation des roches mètres, de leur potentiel pétrolier et de leur degré d'évolution. Rev. Inst. Fr. Pétrol. 32 (1), 23–42.
Farmer, V., Mitchell, B., 1963. Occurrence of oxalates in soil clays following hydrogen peroxide treatment. Soil Sci. 96 (4), 221–229.
Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450 (7167) 277-U210.
Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., Cadule, P., Doney, S., Eby, M., Fung, I., Bala, G., John, J., Jones, C., Joos, F., Kato, T., Kawamiya, M., Knorr, W., Lindsay, K., Matthews, H.D., Raddatz, T., Rayner, P., Reick, C., Roeckner, E., Schnitzler, K.G., Schnur, R., Strassmann, K., Weaver, A.J., Yoshikawa, C., Zeng, N., 2006. Climate–carbon cycle feedback analysis: results from the C4MIP model intercomparison. J. Clim. 19 (14), 3337–3353.
Gregorich, E.G., Gillespie, A.W., Beare, M.H., Curtin, D., Sanei, H., Yanni, S.F., 2015. Evaluating biodegradability of soil organic matter by its thermal stability and chemical composition. Soil Biol. Biochem. 91, 182–191.
Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., Ludwig, B., 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. Eur. J. Soil Sci. 58 (6), 1316–1329.
Helfrich, M., Flessa, H., Dreves, A., Ludwig, B., 2010. Is thermal oxidation at different temperatures suitable to isolate soil organic carbon fractions with different turnover? J. Plant Nutr. Soil Sci. 173 (1), 61–66.
Kleber, M., Mikutta, R., Torn, M.S., Jahn, R., 2005. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. Eur. J. Soil Sci. 56 (6), 717–725.
Knorr, W., Prentice, I., House, J., Holland, E., 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433 (7023), 298–301.
Kögel-Knabner, I., 2017. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: fourteen years on. Soil Biol. Biochem. 105, A3–A8.
Lafargue, E., Marquis, F., Pillot, D., 1998. Rock-Eval 6 applications in hydrocarbon exploration, production, and soil contamination studies. Rev. Inst. Fr. Pétrol. 53 (4), 421–437.
LaRowe, D.E., Van Cappellen, P., 2011. Degradation of natural organic matter: a thermodynamic analysis. Geochim. Cosmochim. Acta 75 (8), 2030–2042.
Lefevre, R., Barre, P., Moyano, F.E., Christensen, B.T., Bardoux, G., Eglin, T., Girardin, C., Houot, S., Kaetterer, T., Oort, F., 2014. Higher temperature sensitivity for stable than for labile soil organic carbon–evidence from incubations of long-term bare fallow soils. Glob. Chang. Biol. 20 (2), 633–640.
Leifeld, J., Kögel-Knabner, I., 2001. Organic carbon and nitrogen in fine soil fractions after treatment with hydrogen peroxide. Soil Biol. Biochem. 33 (15), 2155–2158.
Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., Chen, J., Yang, X., Li, B., 2013. Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. Ecology 94 (3), 726–738.
Lutfalla, S., Chenu, C., Barré, P., 2014. Are chemical oxidation methods relevant to isolate a soil pool of centennial carbon? Biogeochemistry 118 (1–3), 135–139.
Marañón-Jiménez, S., Soong, J.L., Leblans, N.I., Sigurdsson, B.D., Peñuelas, J., Richter, A., Asensio, D., Fransen, E., Janssens, I., 2018. Geothermally warmed soils reveal persistent increases in the respiratory costs of soil microbes contributing to substantial C losses. Biogeochemistry 1–16.
Menichetti, L., Houot, S., van Oort, F., Kätterer, T., Christensen, B., Chenu, C., Barré, P., Vasilyeva, N., Ekblad, A., 2015. Increase in soil stable carbon isotope ratio relates to loss of organic carbon: results from five long-term bare fallow experiments. Oecologia 177 (3), 811–821.
Mikutta, R., Kleber, M., Jahn, R., 2005a. Poorly crystalline minerals protect organic carbon in clay subfractions from acid subsoil horizons. Geoderma 128 (1–2), 106–115.
Mikutta, R., Kleber, M., Kaiser, K., Jahn, R., 2005b. Review: organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. Soil Sci. Soc. Am. J. 69 (1), 120–135.
O'Gorman, E.J., Benstead, J.P., Cross, W.F., Friberg, N., Hood, J.M., Johnson, P.W., Sigurdsson, B.D., Woodward, G., 2014. Climate change and geothermal ecosystems: natural laboratories, sentinel systems, and future refugia. Glob. Chang. Biol. 20 (11), 3291–3299.
Ohno, T., Parr, T.B., Gruselle, M.C.I., Fernandez, I.J., Sleighter, R.L., Hatcher, P.G., 2014. Molecular composition and biodegradability of soil organic matter: a case study comparing two new England Forest types. Environ. Sci. Technol. 48 (13), 7229–7236.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. vegan: Community Ecology Package. R Package Version 1.17-2. R Foundation for Statistical Computing, Vienna Available: CRAN. R-project. org/package = vegan.(July 2012).
Plante, A.F., Fernández, J.M., Leifeld, J., 2009. Application of thermal analysis techniques in soil science. Geoderma 153 (1–2), 1–10.
Poeplau, C., Don, A., 2013. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma 192 (0), 189–201.
Poeplau, C., Don, A., 2014. Soil carbon changes under Miscanthus driven by C4 accumulation and C3 decomposition–toward a default sequestration function. GCB Bioenergy 6 (4), 327–338.
Poeplau, C., Kätterer, T., Leblans, N.I., Sigurdsson, B.D., 2017. Sensitivity of soil carbon fractions and their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. Glob. Chang. Biol. 23 (3), 1316–1327.
R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rasse, D.P., Dignac, M.F., Bahri, H., Rumpel, C., Mariotti, A., Chenu, C., 2006. Lignin turnover in an agricultural field: from plant residues to soil-protected fractions. Eur. J. Soil Sci. 57 (4), 530–538.
Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126 (4), 543–562.
Saenger, A., Cécillon, L., Sebag, D., Brun, J.-J., 2013. Soil organic carbon quantity, chemistry and thermal stability in a mountainous landscape: a Rock–Eval pyrolysis survey. Org. Geochem. 54, 101–114.
Saenger, A., Cécillon, L., Poulenard, J., Bureau, F., De Daniéli, S., Gonzalez, J.-M., Brun, J.-J., 2015. Surveying the carbon pools of mountain soils: a comparison of physical fractionation and Rock-Eval pyrolysis. Geoderma 241, 279–288.
Schiedung, M., Don, A., Wordell-Dietrich, P., Alcántara, V., Kuner, P., Guggenberger, G., 2017. Thermal oxidation does not fractionate soil organic carbon with differing biological stabilities. J. Plant Nutr. Soil Sci. 180 (1), 18–26.
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478 (7367), 49–56.
Schulten, H.-R., Leinweber, P., Theng, B., 1996. Characterization of organic matter in an interlayer clay-organic complex from soil by pyrolysis methylation-mass spectrometry. Geoderma 69 (1–2), 105–118.
Sebag, D., Verrecchia, E.P., Cécillon, L., Adatte, T., Albrecht, R., Aubert, M., Bureau, F., Cailleau, G., Copard, Y., Decaens, T., Disnar, J.R., Hetényi, M., Nyilas, T., Trombino, L., 2016. Dynamics of soil organic matter based on new Rock-Eval indices. Geoderma 284, 185–203.
Sigurdsson, B.D., Leblans, N.I., Dauwe, S., Gudmundsdottir, E., Gundersen, P., Gunnarsdottir, G.E., Holmstrup, M., Ilieva-Makulec, K., Katterer, T., Marteinsdottir, B.-S., 2016. Geothermal ecosystems as natural climate change experiments: the ForHot research site in Iceland as a case study. Icel. Agric. Sci. 29, 53–71.
Siregar, A., Kleber, M., Mikutta, R., Jahn, R., 2005. Sodium hypochlorite oxidation reduces soil organic matter concentrations without affecting inorganic soil constituents. Eur. J. Soil Sci. 56 (4), 481–490.
Six, J., Conant, R., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241 (2), 155–176.
Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74 (1–2), 65–105.
Soucémarianadin, L., Cécillon, L., Chenu, C., Baudin, F., Nicolas, M., Girardin, C., Barré, P., 2018. Is Rock-Eval 6 thermal analysis a good indicator of soil organic carbon lability?–a method-comparison study in forest soils. Soil Biol. Biochem. 117, 108–116.
Spohn, M., Giani, L., 2011. Total, hot water extractable, and oxidation-resistant carbon in sandy hydromorphic soils-analysis of a 220-year chronosequence. Plant Soil 338 (1), 183–192.
Sykes, R., Snowdon, L.R., 2002. Guidelines for assessing the petroleum potential of coaly source rocks using Rock-Eval pyrolysis. Org. Geochem. 33 (12), 1441–1455.
Taghizadeh-Toosi, A., Christensen, B.T., Hutchings, N.J., Vejlin, J., Kätterer, T., Glendining, M., Olesen, J.E., 2014. C-TOOL: a simple model for simulating whole-profile carbon storage in temperate agricultural soils. Ecol. Model. 292, 11–25.
von Lützow, M., Kogel-Knabner, I., Ekschmittb, K., Flessa, H., Guggenberger, G., Matzner,

E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39 (9), 2183–2207.
Westerhoff, P., Chao, P., Mash, H., 2004. Reactivity of natural organic matter with aqueous chlorine and bromine. Water Res. 38 (6), 1502–1513.
Wickham, H., 2015. stringr: Simple, Consistent Wrappers for Common String Operations. R Package Version 1.0.0.
Wild, B., Schnecker, J., Alves, R.J.E., Barsukov, P., Bárta, J., Čapek, P., Gentsch, N., Gittel, A., Guggenberger, G., Lashchinskiy, N., Mikutta, R., Rusalimova, O., Šantrůčková, H., Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., Richter, A., 2014. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. Soil Biol. Biochem. 75, 143–151.
Xie, G., Barcelona, M.J., 2003. Sequential chemical oxidation and aerobic biodegradation of equivalent carbon number-based hydrocarbon fractions in jet fuel. Environ. Sci. Technol. 37 (20), 4751–4760.
Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007. Measured soil organic matter fractions can be related to pools in the RothC model. Eur. J. Soil Sci. 58 (3), 658–667.